1.02, 27.0, 33.8

PERFORMANCE REPORT

RESEARCH AND SURVEYS

FEDERAL AID PROJECT NO. F-39-R-16

MICROPTERUS BASS INVESTIGATIONS

JOB NO. 12

EVALUATION OF GENETIC STATUS OF OKLAHOMA LARGEMOUTH BASS POPULATIONS

MARCH 1, 1993 through FEBRUARY 28, 1994

PERFORMANCE REPORT

STATE: Oklahoma PROJECT NUMBER: F-39-R-16

PROJECT TITLE: Micropterus Bass Investigations

JOB NUMBER: 12

JOB TITLE: Evaluation of Genetic Status of Oklahoma Largemouth Bass Populations

I. PROGRAM NARRATIVE OBJECTIVE NO. 12:

To electrophoretically evaluate the genetic impact of Florida largemouth bass introductions on existing populations of northern largemouth bass in selected Oklahoma lakes and reservoirs.

II. JOB SEGMENT OBJECTIVE:

To electrophoretically evaluate the genetic impact of Florida largemouth bass introductions on existing populations of northern largemouth bass in selected Oklahoma lakes and reservoirs.

III. SUMMARY OF PROGRESS:

A. Methods

Trophy Bass

Cooperating Oklahoma taxidermists were asked to save liver and scale samples from bass >3.6 kg for phenotype and age determination of trophy bass caught from public waters.

Stream Sampling

Stream sampling methods are explained in Appendix 1.

Experimental Stockings

Longmire and Bristow lakes were stocked in 1993 for the fourth consecutive year with Florida largemouth bass (FLMB) fingerlings (mean TL = 37 mm @ 247/ha). Lake Thunderbird received 111 advanced FLMB fingerlings (mean TL = 228 mm) and 1,782 fingerlings (mean TL = 75 mm) via a nursery pond. Lakes Crowder-SE and Onapa received FLMB fry (mean TL = 15 mm @ 2470/ha) for the third year and Bixhoma Lake received fry for the

second year. Spring and fall samples of bass were collected by electrofishing from each of the above lakes following procedures described by Gilliland and Whitaker (1989). Fall samples were collected from nine lakes stocked from 1988 through 1991 (Caddo, Carter, Chambers, Chelsea, Crowder, El Reno, Pawnee, Watonga, and Wetumka).

B. Results

Trophy Bass

Tissue and scale samples from 63 trophy bass (3.6-5.8 kg) caught by anglers in 1993 from 26 different public waters were analyzed. Fifteen percent of these trophies were northern largemouth bass (NLMB; n = 9), 53% were FLMB (n = 35), 32% were INTLMB (intergrades phenotypes - F_1 or F_x, n = 19; Table 1). Ages of these bass determined from scales or otoliths were from 6 to 12 years. Letters reporting the phenotype and age of their trophy bass were sent to each contributing angler, and summaries were sent to each cooperating taxidermist. A manuscript and presentation describing five years of data collection using a taxidermist network was prepared for the Southeastern Association of Fish and Wildlife Agencies annual conference in 1993. The Oklahoma "Top Twenty" bass list was updated using entries from the ODWC Angler Recognition Program (ARP).

Stream Sampling

Results of stream sampling are explained in Appendix 1. A manuscript detailing the introgression of FLMB alleles into Oklahoma stream bass populations was prepared and submitted for

consideration in the Transactions of the American Fisheries Society. Another manuscript concerning the genetic variability among populations of largemouth bass in Oklahoma streams was submitted to Southwestern Naturalist.

Experimental Stockings

When fall 1992 samples of largemouth bass from the four lakes stocked that year were compared to spring bass samples in 1993, the proportion of a year class made up of stocked FLMB generally declined (Table 2). Percentages of FLMB in fall 1992 samples from Arcadia declined from 10% to 0% the following spring. The %FLMB at Bristow declined from 10% in fall 1992 and 8% in spring 1993; at Longmire from 22% to 0%; at Crowder-SE from 34% to 13%. The %FLMB increased from 28% to 32% at Onapa.

Fall sampling indicated survival of FLMB stocked in 1993 was variable (Table 2). The 1993 year class at Thunderbird was comprised of 0% FLMB (but had 49% INTLMB), Crowder-SE 15%, Onapa 25%, Bristow and Longmire 41% each.

Age-0 bass were collected at Caddo, Carter, Chambers, Chelsoma, Crowder, El Reno, Pawnee, Watonga and Wetumka lakes where FLMB were stocked in each for three consecutive years between 1988 to 1991. The 1993 year classes contained no pure FLMB offspring in any of these lakes (Table 2). Pure FLMB collected from Chambers were probably the result of an introduction of fingerlings the week prior to sampling. These FLMB were significantly smaller than the NLMB in the sample. Intergrade phenotypes made up 6% of the age-0 bass in Caddo, 14%

in Carter, 0% in Chambers, 13% in Crowder, 16% in Chelsoma, 21% in El Reno, 33% in Pawnee, 9% in Watonga, and 28% in Wetumka.

IV. Significant Deviations:

Statistically reliable samples of age-0 bass ($n \geq 30$) were not obtained in spring 1993 from Arcadia due to high water conditions. Low bass densities in El Reno, Pawnee, and Watonga also made collecting sufficient samples impractical.

V. Conclusions, Evaluations and Recommendations:

Trophy Bass

Fewer trophy bass samples were collected from cooperating taxidermists in 1993 ($n = 63$) than in previous years (down from an average of 75/year over five years). Although more taxidermists participated during the year, we received fewer samples from each. This decline in trophy bass numbers may be attributed to poor weather conditions and extremely high lake levels during the peak spring trophy bass season resulting in lower angler effort and fewer big bass being caught. Increased voluntary release of trophy bass by anglers may have also contributed to this decline. Low application rates for ARP Trophy Fish Conservationist awards also prevented collecting data on trophies that were caught and released. Another factor contributing to this decline may be the continued decline in numbers of pure FLMB and F_1 hybrids originally stocked into in many reservoirs. Stockings of "FLMB" in the 1970's and early 1980's were not continued and the trophy fish that resulted have essentially left those systems through natural or fishing

mortality. It will be several more years before benefits from revived stocking programs started in the 1990's are seen.

Experimental Stockings

Following the pattern established in previous years, survival of stocked FLMB to fall 1993 was variable with these fish making up from 0% to 74% of their respective year classes (Table 2). Survival of FLMB from age 0 (fall 1992) to age 1 (spring 1993) was also mixed, with over-winter mortality ranging from 0% at Onapa, 20% at Bristow, 62% at Crowder-SE, and 100% at Arcadia and Longmire. No mortality estimate was made for Thunderbird because a fall 1992 sample was not collected.

Fingerlings from the USFWS stocked in 1993 tested 95% FLMB (by electrophoresis). Age-0 F_1 and/or F_x INTLMB were found in fall 1993 samples from Arcadia (2%), Bristow Lake (23%) and Longmire (15%; Table 2). These percentages of non-FLMB phenotypes were higher than those found in the stocked fish. This indicated that FLMB stocked in 1990 and 1991 had reached sexual maturity and that reproduction had taken place among these fish and the native NLMB or other INTLMB in the populations, or that adult INTLMB from other sources in the watershed migrated into the lake contributing to F_x production. High percentages of F_1 INTLMB at Onapa (40%) were from matings among the maturing age-2 FLMB and native NLMB in the lake.

Recommendations

Trophy Bass

Obtaining tissue and scale samples through cooperating taxidermists should continue. Distribution of Angler Recognition Program posters should continue statewide to inform anglers about the value of data from trophy bass. All biologists should emphasize participation in the ARP when making presentations to bass clubs and angler groups.

Experimental Stockings

Stockings of FLMB fry should be made as scheduled in 1994 for Bixhoma Lake. Fingerling or advanced fingerling stockings should be repeated at lakes Arcadia, Longmire, and Thunderbird. Continued sampling of all FLMB populations using spring and/or fall electrofishing is recommended to determine 1) the contribution stocked fish are making to each year class, 2) when differential mortality appears to occur, and 3) to what degree introgression of FLMB alleles is occurring within these populations.

All work and subsequent Performance and Final reports formerly contracted under Project F-39-R, Job 12 will be continued as F-50-R, Project 1.

IV. Prepared by: Gene Gilliland

Gene Gilliland, Biologist II

V. Date: May 25, 1994

VI. Approved by: Harold Namminga

Dr. Harold Namminga, Federal Aid Research Coordinator

VII. Literature Cited

Gilliland, G. and J. Whitaker. 1989. Evaluation of genetic status of largemouth bass populations. Oklahoma Department Wildlife Conservation, Federal Aid Project F-39-R, Job 12, Performance Report, Oklahoma City.

Table 1. Numbers and phenotypes, by lake, of trophy largemouth bass (≥ 3.6 kg), caught from Oklahoma public waters in 1993 (provided by taxidermists).

LAKE	WEIGHT	NLMB	FLMB	F_1	F_x
Arbuckle	8 - 10 lbs.	0	0	1	0
Comanche	> 10 lbs.	0	0	1	0
Dripping Spgs.	8 - 10 lbs.	0	0	1	0
Ellsworth	8 - 10 lbs.	1	0	0	0
Ft. Gibson	8 - 10 lbs.	1	0	0	0
Fuqua	8 - 10 lbs.	0	1	1	0
Grand	8 - 10 lbs.	1	0	0	0
Holdenville	8 - 10 lbs.	0	0	1	0
Hugo	> 10 lbs.	0	1	1	0
Keystone	8 - 10 lbs.	1	0	0	0
Konawa	8 - 10 lbs.	1	1	0	0
Lawtonka	> 10 lbs.	0	1	0	0
Lone Chimney	> 10 lbs.	1	0	0	0
McGee Creek	8 - 10 lbs.	0	6	1	0
	> 10 lbs.	0	2	0	0
Mountain Lake	> 10 lbs.	0	2	3	0
Murray	8 - 10 lbs.	0	1	0	0
	> 10 lbs.	0	0	1	0
Ozzie Cobb	8 - 10 lbs.	0	0	1	0
Pine Creek	8 - 10 lbs.	0	0	1	0
Pittsburg	8 - 10 lbs.	1	0	0	0
Purcell	8 - 10 lbs.	1	0	0	0
Raymond Gary	8 - 10 lbs.	0	1	0	0
Sardis	8 - 10 lbs.	0	8	2	0
	> - 10 lbs.	0	11	1	0
Scott King	> 10 lbs.	0	0	1	0
Skiatook	8 - 10 lbs.	0	0	1	0
Watonga	8 - 10 lbs.	0	0	1	0
Wister	8 - 10 lbs.	1	0	0	0

Table 2. Phenotypes of largemouth bass collected from 19 Oklahoma lakes stocked with Florida largemouth bass 1987-1993; collection dates; sample size by age group (N); number (#) and percent (%) of NLMB, FLMB, INTLMB (F1, Fx, total intergrades), and bass with any FLMB alleles.

Lake Date	Age	N	NLMB #(%)	FLMB #(%)	INTLMB #(%)F_1/#(%)F_x/#(%)INT	ANYFLMB #(%)
Arcadia						
10/92	0	30	16(53)	3(10)	2(7)/ 9(30)/11(37)	14(47)
5/93	1	11	6(55)	0(0)	2(18)/ 3(27)/ 5(45)	5(45)
	2	7	6(86)	0(0)	0(0)/ 1(14)/ 1(14)	1(14)
10/93	0	38	9(24)	28(74)	0(0)/ 1(2)/ 1(2)	29(76)
Bixhoma						
4/92	1	40	20(50)	20(50)	0(0)/ 0(0)/ 0(0)	20(50)
10/93	0	90	40(44)	27(30)	23(26)/ 0(0)/23(26)	50(56)
Bristow						
10/92	0	30	9(30)	3(10)	8(27)/10(33)/18(60)	21(70)
4/93	1	36	24(67)	3(8)	7(19)/ 2(5)/ 9(25)	12(33)
	2	21	9(43)	0(0)	8(38)/ 4(19)/12(57)	12(57)
10/93	0	39	14(36)	16(41)	8(21)/ 1(2)/ 9(23)	25(64)
Caddo						
10/92	1	30	27(90)	0(0)	0(0)/ 3(10)/ 3(10)	3(10)
10/93	1	32	30(94)	0(0)	0(0)/ 2(6)/ 2(6)	2(6)
Carter						
10/92	0	33	31(94)	0(0)	2(6)/ 0(0)/ 2(6)	2(6)
10/93	0	36	31(86)	0(0)	0(0)/ 5(14)/ 5(14)	5(14)
Chambers						
10/92	0	18	13(72)	0(0)	0(0)/ 5(18)/ 5(18)	5(18)
10/93	1	48	42(88)	8(12)	0(0)/ 0(0)/ 0(0)	8(12)
Chelsoma						
10/92	0	30	25(83)	0(0)	5(17)/ 0(0)/ 5(17)	5(17)
10/93	0	55	46(84)	0(0)	0(0)/ 9(16)/ 9(16)	9(16)
Crowder						
10/92	0	30	22(73)	0(0)	1(4)/ 7(23)/ 8(27)	8(27)
10/93	0	40	33(87)	0(0)	0(0)/ 7(13)/ 7(0)	7(13)

Table 2. Continued.

Lake Date	Age	N	NLMB #(%)	FLMB #(%)	INTLMB #(%)F_1/#(%)F_x/#(%)INT	ANYFLMB #(%)
Crowder-SE						
10/92	0	32	14(44)	11(34)	2(6)/ 5(16)/ 7(22)	18(54)
5/93	1	24	20(82)	3(13)	0(0)/ 1(5)/ 1(5)	4(18)
	2	16	16(100)	0(0)	0(0)/ 0(0)/ 0(0)	0(0)
10/93	0	40	34(85)	6(15)	0(0)/ 0(0)/ 0(0)	6(15)
El Reno						
10/92	0	30	21(70)	0(0)	3(10)/ 6(20)/ 9(30)	9(30)
10/93	0	14	11(79)	0(0)	1(7)/ 2(14)/ 3(21)	3(21)
Longmire						
10/92	0	23	8(35)	5(22)	9(39)/ 1(4)/10(43)	15(67)
5/93	1	46	33(70)	3(0)	7(10)/ 3(20)/10(30)	13(30)
	2	40	34(85)	2(6)	3(8)/ 1(2)/ 4(10)	6(15)
10/93	0	71	31(44)	29(41)	4(6)/ 7(9)/11(15)	40(56)
Onapa						
10/92	0	32	21(66)	9(28)	1(3)/ 1(3)/ 2(6)	11(34)
5/93	1	31	20(65)	10(32)	1(3)/ 0(0)/ 1(3)	11(35)
10/93	0	40	14(35)	10(25)	16(40)/ 0(0)/16(40)	26(65)
Pawnee						
10/92	0	23	17(74)	0(0)	1(4)/ 5(22)/ 6(26)	6(26)
10/93	0	27	18(67)	0(0)	0(0)/ 9(33)/ 9(33)	9(33)
Thunderbird (via nursery pond)						
10/93	0	64	33(51)	0(0)	6(9)/25(39)/31(49)	31(49)
Watonga						
10/92	0	32	30(94)	0(0)	0(0)/ 2(6)/ 2(6)	2(6)
10/93	0	11	10(91)	0(0)	0(0)/ 1(9)/ 1(9)	1(9)
Wetumka						
10/92	0	31	14(48)	0(0)	10(32)/ 6(20)/16(52)	16(52)
	1	19	11(58)	0(0)	4(21)/ 4(21)/ 8(42)	8(42)
10/93	0	25	18(72)	0(0)	0(0)/ 7(28)/ 7(28)	7(28)

Appendix 1.

Evaluation of Genetic Status of Stream Populations of Largemouth Bass in Oklahoma

Report by Frances P. Gelwick

F-39-R, Job 12, Segment 16 (Procedures 10 and 11)

Introduction

The objectives of this study were (1) to determine the allele frequencies at the sAAT-B*, sIDHP*, and sSOD* loci, for evaluation of the possible contribution of introduced Florida largemouth bass (Micropterus salmoides floridanus) to native stream largemouth bass (M. s. salmoides) populations (2) to compare the distribution of Florida-specific alleles with respect to previous studies of reservoir populations by Gilliland and Whitaker (1989) and Gilliland (In Press) and (3) to determine the genetic variation among stream largemouth bass (M. salmoides) across Oklahoma based on the above loci plus three additional loci: sMDH-A*, sMDH-B*, PGM* and GPI-A*, GPI-B*, previously shown to be variable in populations across the United States (Phillip et al. 1982). Nomenclature of loci and alleles follows that of Shaklee et al. (1990).

Methods

In 1993 and 1994 seining collections for largemouth bass were made at 144 sites across 21 Oklahoma stream drainages (Figure 1). Additional fish were provided for this study from 21 sites by P. Moershell (Oklahoma Water Resources Board), from 16 sites by W. J. Matthews (University of Oklahoma Biological Station) and from 2 sites by C. Taylor and M. Pyron (University of Oklahoma Biological Survey). Primarily young-of-year fish were analyzed (although older fish were also examined, i.e. 54 fish > 150 mm), totaling 610 fish from 161 sites. Fish were kept on ice in the field and then frozen until tissues were extracted.

Distribution of bass with Florida-subspecific alleles were compared to the results of Gilliland and Whitaker (1989) and Gilliland (In Press) for survival of stocked Florida largemouth bass, especially with respect to the annual cumulative heating degree days (HDD; the sum over all days fall to spring of the difference between 18.3°C and the average daily temperature).

All laboratory procedures were carried out at the Oklahoma Fisheries Research Laboratory, Norman. Additional tissue samples (liver, muscle and eye) were archived in an ultra-cold freezer in the Zoology Department at the University of Oklahoma. Known Florida largemouth bass liver tissues were analyzed as controls on all gels for the detection of Florida subspecific alleles. Muscle tissue extracts were used to detect alleles for the three additional loci examined. All enzymes were run in Tris-citrate buffer systems. Alleles were numbered according to their migration speed; those alleles migrating further anodally from the origin were given greater numerical designation and follows Phillip et al. (1982). Cross-identification of allele migration patterns with known fish from other laboratories was not done; variation among laboratories with respect to methods and materials is generally unknown. Therefore, only relative similarities and differences of alleles at these loci could be compared to those previously published.

For the 610 fish analyzed, 19 alleles were scored at 8 loci. From the genotype data, the variability within populations was calculated as the percentage of polymorphic loci as well as the mean observed and expected heterozygosity (Nei 1978). The program BIOSYS-1 (Swofford and Selander 1981) was used to calculate (1) estimated variance components among populations,

i.e. σ^2, and (2) relatedness (correlation) of alleles within individuals among populations, i.e. fixation statistics (F_{xy}, Wright 1978). These statistics reflect the nesting of minor drainage populations within the hierarchy of four large river systems within the two major river drainages (Arkansas and Red) in Oklahoma. The gene frequencies were used to construct matrices of genetic identity (Nei 1978). Unweighted-pair-group-cluster analysis (UPGMA) and principal coordinates ordination (PCORD) using the program NTSYS-pc (Rohlf 1990) were used to analyze multivariate relationships among all drainage populations based on the genetic identity matrix.

Results and Discussion

The distribution of sites surveyed and percentages of sites with largemouth bass and with Florida alleles is shown in Table 1. Distribution of largemouth bass was similar across drainages; approximately 60 percent of sites contained bass. However, the distribution of Florida bass generally followed a geographic pattern for survival of experimentally stocked Florida bass in Oklahoma reservoirs previously documented by Gilliland and Whitaker (1989) and Gilliland (In Press). Florida alleles were detected in more collections southeast than northwest of the 3400 HDD cline (Table 1). The relative abundance of fish with at least one Florida allele ranged from 1.5% (of 203 total fish) from drainages in the northwest half of the state to 5.2% (of 407 total fish) in the southeast (Table 1).

Allele Frequencies

Average percentage contribution of Florida alleles was higher in the southeast than in the northwest half of the state (Table 2). Allele frequencies at these loci for non-intergrade

populations were similar to frequencies found for populations from Grand and Texoma lakes by Phillip et al. (1982).

The majority of fish (17 of 24 total) with Florida alleles were considered most likely to be F_x intergrades (second or subsequent generation hybrids, Table 3). The genotype of one individual indicated full Florida sub-specific status. It was 89 mm T.L. when collected in late summer in the Little River near Wright City downstream of Pine Creek Reservoir. It should be noted that matings between F_x individuals may result in random combinations of alleles that would be mis-identified as pure Florida bass using these diagnostic loci. Only one other individual with Florida alleles (an F_x intergrade) was collected from this drainage.

Five of the six F_1 intergrades (first generation hybrid northern x Florida bass) were collected at one site (West Cache Creek, Table 3) and were < 96 mm TL when collected in November. The nearest stocking of Florida bass to this site was Lake Lawtonka in the East Cache Creek drainage. However, Florida bass were stocked by personnel at the Fort Sill Military Reservation. These bass were likely stocked into earthen ponds which may overflow during high water events (L. Cofer, personal communication) and drain into these tributaries.

Two individuals from the Baron Fork drainage had sIDH*2 alleles similar to those found in spotted bass (M. punctulatus). Because spotted bass were found with largemouth bass in these collections, and because a slower migrating allele between sIDH*1 and sIDH*3 is not known in largemouth bass, the possibility of hybridization is most likely. Because these individuals showed some morphological resemblance to spotted bass (cecae counts),

they were not included in these analyses. One rare allele (sIDH*0) was detected in two collections (Blue and Boggy rivers, one fish each). This allele appears to be similar to that identified in largemouth bass as sIDH*0 by Hallerman et al. (1986) and Norgren et al. (1988).

Allele frequencies for the additional loci are shown in Table 4. At each of two loci (sMDH-A* and GPI-A*) only a single allele was found in all individuals examined. This followed findings in populations from Grand Lake and Lake Texoma by Phillip et al. (1982). Allele frequencies for PGM were similar to those for Grand and Texoma lakes (Phillip et al. 1982). However, frequencies for sMDH-B* were variable and did not show a differentiation between the two major river drainages as might be inferred from the results for Grand and Texoma lakes (Phillip et al. 1982). In contrast, results for GPI-B* were more similar between the two major drainages than the previous study showed for Grand and Texoma, but all results consistently show higher frequencies for the slower GPI-B* allele.

The sMDH-B*3 allele was found in three individuals from the Baron Fork drainage. This is a fast migrating allele similar to that identified in smallmouth bass (M. dolomieui). Although, the morphology of these fish did not indicate hybridization, both species of bass were collected together in this river. Further electrophoretic analyses of these tissue samples against known smallmouth individuals for other loci would be necessary to determine if this allele is a result of interspecific hybridization or a new rare allele for largemouth bass.

Loci not conforming to Hardy-Weinberg Equilibrium (HWE) were found in five drainages (Tables 2 and 4). Such departures

indicate that samples may represent populations that are not large or mating at random, or that forces such as selection or migration are acting. Most departures from HWE were detected at Florida-subspecific loci. Florida alleles were detected in four of five drainages. When the bass with Florida alleles were removed from the data set, only departures at non-Florida loci remained.

Not all populations with Florida alleles showed departure from HWE; Cache Creek, with the highest average Florida allele frequency conformed to HWE expectations, indicating that little selection against these alleles is occurring in this population. However, failure to detect departures from HWE does not necessarily indicate that all assumptions of HWE hold (Weir 1990).

Departures from HWE also could be due to segregation of different gene pools within the drainage if movement of bass within the drainage is limited, or if more than one subpopulation is included as one population. Departures from HWE also could occur if two populations interbreed to some extent but mate assortatively within populations (Chakraborty and Leimar 1987, Campton 1987). The previously noted conditions usually lead to a deficiency of heterozygotes in comparison to that expected under HWE. Heterozygote deficiency was found in all cases of non-HWE in this study.

Genetic variability

Three measures of genetic variability at the 8 loci are shown in Table 5. The addition of Florida bass alleles to a population increases the total number of alleles in the gene pool. Because the distribution of Florida alleles was primarily

in the southeast region of Oklahoma, this region would be expected to have higher mean number of alleles, higher percentage of polymorphic loci and higher mean heterozygosity. Thus, it is logical that the mean for these measures was yet higher when only those drainages containing Florida bass were included (Table 4).

Genetic differentiation among drainage populations is shown in Table 6. Much of the genetic information was due to individual drainage populations. The highest genetic variability (variation) was seen when both the minor drainage population and the major river drainage (Arkansas or Red) were considered. However, knowing only the minor drainage population membership was almost as informative. Aggregating drainage populations into their larger river or major river membership decreased the variance, i.e. at the level of large rivers or major drainages allele frequencies were similar.

Note that negative values do not indicate negative variance, but are the result of a mathematical difference between two positive variances (Cocherham 1973, Wright 1978, Weir 1990). This also results from the assumption, for computational purposes, that estimated total variance is partitioned orthogonally into estimated variance components. Thus, if the sum of a part of the estimated variance components exceeds the estimated total variance, the remaining estimated variance components become negative.

A matrix of genetic identity (Nei 1978) among all minor drainage populations (using all 8 loci) showed a range in values from 1.000 to .949. Results of averaging these scores by large river system and major river drainage is shown in Tables 7a and 7b. These results were generally higher than similar comparisons

reported by Phillip et al. (1982) of individual populations across the nation (ranging 1.000 to .880), or averaged by stock (northern, Florida and intergrade; ranging .997 to .911). Although fewer loci were used in this study (as compared to 28 loci of Phillip et al. [1982]), those identifying the Florida alleles, which contributed to much of the geographic variability among stocks, were common to both studies. The Oklahoma drainage populations appeared to be a more homogeneous group, as might be expected of a regional sub-sample from nation-wide populations with demonstrated latitudinal gradients in frequencies of these alleles.

Among the four major rivers of Oklahoma, the lowest identity (greatest heterogeneity) occurred within the Red River west (upstream) of Lake Texoma (0.983, Table 7a). This area contained the highest average Florida allele frequencies (Table 2). The overall lowest identity resulted when the Red River west was averaged with the Red River east (downstream) of Lake Texoma (0.983, Table 7a). When populations were averaged over major river drainages (Table 7b) the identity values within and between groups were higher (indicating less heterogeneity). This agrees with the pattern of genetic variance shown in Table 6, and indicates that most of the heterogeneity among minor drainage populations was found within the Red River.

Multivariate Analyses

The results of the cluster and ordination analyses complement each other; the cluster analysis best indicates relationships within major groups (towards the tips of the cluster) and the ordination best indicates relationships among larger groups. These analyses identified two major groups among

the drainage populations (Figures 2 and 3). The smaller group is made up of four drainages, two from the main Arkansas and one each from the Canadian and Red River east of Lake Texoma. The larger group is made up of 12 drainages, four from the Red River west (upstream) of Lake Texoma and two from the Red River east (downstream) of Lake Texoma, and three each from the Canadian and main Arkansas drainages. The remaining five drainage populations do not group closely among themselves or with any other group. Two of each of these drainage populations are from the main Arkansas and Red River west of Lake Texoma, and the fifth is from the Red River east of Lake Texoma. Thus, there are no strong drainage or larger river patterns. These results are similar to those of the genetic variability analysis (Tables 6, 7a and b).

The three-dimensional plot of the first three axes of the principal coordinates analysis shows the relationships among the clustering groups (Figure 3). The strength of the first two axes were similar (eigenvalues of 4.3 and 3.9, respectively) and the third axis was almost half as strong as the first two (eigenvalue of 2.1). The numbers in parentheses in Figure 3 indicate the scale of correlation of each drainage population along that axis. Each allele is listed towards the end of the axis with which it is most highly correlated. Thus, the Illinois drainage population was characterized by higher frequency of sIDH*0 and sAAT*2 on axis 1, of sMDH-B*3 and PGM*4 on axis 2 and of GPI*3 on axis 3. It was also evident that the three Florida alleles (sIDH*3, sAAT*3/4) are strongly intercorrelated, i.e. all loaded highly on axis 1. Thus, drainage populations with more Florida alleles are found further to the left on axis 1. A minimum spanning tree was calculated to determine the shortest Euclidian

distance between all populations based on genetic similarity (Table 8). A portion of this tree has been imposed on the ordination to indicate the nearest neighbors to drainages not included in either of the two clusters, e.g. Cache Creek is most similar to Blue River and Cimarron is most similar to the Salt Fork of the Arkansas.

Management Implications

The contribution of Florida alleles to native stream largemouth bass populations was variable with respect to number and geographic distribution of sites and number of individuals among drainages. However, presence of Florida alleles generally followed a northwest to southeast gradient of increasing contribution as was found in reservoir populations by Gilliland (In Press). Much of the genetic variability among stream populations was located within drainages of the Red River and probably due in part to the presence of Florida alleles. Most of this variability occurred at the level of minor drainages, indicating the distinctiveness of each drainage. Variability overall among minor drainage populations was slightly lower than that among largemouth bass populations across the nation (Phillip et al. 1982). However, this observation is inconclusive in that this study examined only 8 loci in comparison to the 28 loci examined by Phillip et al. (1982).

Although low rates of introgression of Florida largemouth bass alleles were found in the majority of streams surveyed, in one system (Cache Creek) a mixture of genotypes (F_1 and F_x) and sizes (range 73 mm to 325 mm TL) indicated an admixture of both subspecies (breeding beyond the first generation hybrid stage with little selection occurring). The most likely sources of

these fish were ponds stocked and maintained by U.S. Army personnel at Ft. Sill (L. Cofer, ODWC southwest region, personal communication). In most other cases of streams containing individuals carrying Florida alleles, the source of introgression was likely not reservoirs stocked by ODWC, due to distance from such sources.

A likely explanation for non-HWE is migration into populations (via release or escape), because few of the total bass sampled carried Florida alleles. It appeared however, that ODWC stocking programs were having minimal impact on native stream bass populations. Collection locations of fish that carried Florida alleles were not closely associated (directly upstream or downstream on the same stream) with reservoirs stocked with Florida bass.

It appears that stock transfer by private individuals may be a source of Florida alleles. In the one case where a likely route of introduction from ponds was present, no departures from HWE were found. It cannot be determined if these fish were spawned in the stream. If they were, an admixture may have resulted. It is important that the public understand that ODWC stockings of Florida bass are based on a management strategy with a high probability of producing trophy fishing in reservoirs with a low chance of adverse impacts on native stream populations. Stockings by private individuals generally are not based on any strategy and are usually done for self-serving purposes. Thus, a stronger emphasis in education programs on the impact of such transfers, the role of private individuals in preventing release of such fish into local stream populations, and the discouragement of deliberate or incidental transfer of these fish

by private individuals to public waters is recommended. Enforcement of laws against the introduction of live fish into public waters should be emphasized (Title 29, § 6-504).

It is emphasized that the fish examined were generally young-of-the-year or juveniles which therefore suggests the presence of Florida bass alleles in the active gene pool of populations where they were detected. That is, adult bass carrying Florida alleles are probably present in these populations.

The deficiency of heterozygotes detected in all but one instance at these loci would indicate somewhat segregated subpopulations of Florida and native northern bass. This segregation might occur both spatially (if different habitats were selected by each) or if timing of mating were different between the two subspecies (Isley et al. 1987).

The future impact of introductions of Florida largemouth bass alleles into our populations (both stream and reservoir) is uncertain. However, the greater control ODWC has over such introductions, the more predictable will be the result of any positive or negative effects. Because long-term environmental changes are not predictable, we should not compromise the long-term evolutionary adaptation and success of our native stream-fish populations for a relatively short-term permissive maintenance of introgressed populations. In the event of wide temperature shifts over short (weeks to months) and long (years to decades) time periods (as increasingly predicted by global climate change scenarios), introgression of maladaptive enzyme systems of non-native stock (especially like those of Florida largemouth bass that have evolved in an environment of very low

climatological fluctuation) may theoretically jeopardize the integrity of our native population. This could lead to their depression or depletion just as would overharvesting. At this point the decision to restock and from what population would be important. However, in this report we have identified the location of populations most likely to be influenced at present by non-native Florida largemouth bass alleles, and we have identified the genetic relationships among stream populations based upon alleles at 8 loci. With this information, the ODWC can better judge the genetic influence of stocking and transfer of largemouth bass among streams. This report also identified potential sources of largemouth bass carrying rare alleles (sIDH*0) that my be useful as genetic markers in future stocking programs.

Angler Benefits

The information in this report indicates that it is the private individuals (and anglers) that likely have the greatest influence on introductions in stream largemouth bass populations. The awareness of this impact and knowledge of the uncertainty regarding the ultimate influences of such introductions (helpful and harmful) on stream populations, should influence the angler to allow such management decisions to be made by ODWC and to stop transferring fish of unknown stock among stream populations and discourage others from doing so as well.

Anglers can enjoy fishing for trophy Florida bass in water bodies where they can be maintained appropriately by ODWC, apparently with little threat to native stream populations. Anglers can also benefit by being aware of the information in this report and helping to conserve their locally adapted

populations. These populations are important sources from which to restock Oklahoma streams and reservoirs should some local population be negatively affected by any event (natural or anthropogenic). Fewer angler dollars would be needed to fund restocking, because native stocks likely would be less expensive to maintain than non-native stocks, which would likely require extensive and repeated restocking efforts, especially if they were unable to adapt to local long-term conditions.

Acknowledgments

We thank D. Phillip (Illinois Natural History Survey), J. Koppelman (Missouri Department of Conservation), A. A. Echelle, W. Stark (Oklahoma State University) and J. Grady (University of New Orleans) for discussions of electrophoretic techniques and data analysis. Technical help was contributed by K. Thomas and R. Horton (ODWC). D. Hough, and G. Schnell (Oklahoma Biological Survey) facilitated the use of computer programs used in this analysis. W. Matthews (University of Oklahoma) provided bass from the Wichita Mountain Wildlife Refuge and other sites for inclusion in this study. P. Moershell (Oklahoma Water Resources Board), C. Taylor and M. Pyron (Oklahoma Biological Survey), R. Cashner and students (University of New Orleans), and R. Bastarache, B. Cochran and C. Neihardt (USDA, Forest Service) assisted with seining and provided additional bass from their collections. This research was cooperatively funded by the Oklahoma Biological Survey, Oklahoma Federal Aid to Sport Fish Restoration Project F-39-R, and the Oklahoma Fisheries Research Laboratory. R. Pittman (United States Fish and Wildlife Service) facilitated additional funding for the genetic analysis of bass collected in the Wichita Mountain Wildlife Refuge.

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Table 1. Drainages surveyed for Oklahoma largemouth bass in 1992-1993. Columns indicate the total sites sampled, number of sites with bass (LMB), number of sites where Florida (FLMB) alleles were detected, and number of LMB with Florida alleles. In parentheses, for each major drainage system, region and total sites, are the percentage of LMB sites, average number of bass per site, percentage of LMB sites with FLMB alleles, and percentage of bass with FLMB alleles.

Region[a]/drainage	# Sites sampled	LMB # sites (%)	LMB # bass (#/site)	FLMB # sites (%)	FLMB # bass (#/site)
Arkansas River:					
NW Cimarron	11	6	33	0	0
NW Salt Fork	10	5	22	0	0
NW Verdigris	10	9	27	0	0
NW Neosho	7	6	37	1	1
SE Arkansas tributaries	5	5	32	1	1
SE Illinois	6	4	26	1	3
SE Poteau	16	7	30	1	1
Total	65	42	207	4	6
		(64.6)	(4.9)	(9.5)	(2.9)
Canadian River:					
NW North Canadian (west)	4	3	30	0	0
NW South Canadian (west)	4	3	12	0	0
SE North Canadian (east)	10	8	38	0	0
SE South Canadian (east)	2	2	43	0	0
Total	20	16	123	0	0
		(80.0)	(7.7)	(0.0)	(0.0)
Red River west of Lake Texoma Dam:					
NW Washita (west)	7	5	22	0	0
NW N. Fork and western Red	11	3	20	1	2
SE Washita (east)	9	8	40	1	1
SE Cache and Beaver Creeks	5	4	18	3	10
SE Wichita Mt. Refuge	32	12	54	0	0
SE Brier and Caney Creeks	6	3	14	0	0
Total	70	35	168	5	13
		(50.0)	(4.8)	(14.3)	(7.8)
Red River east of Lake Texoma Dam:					
SE Blue	5	5	18	0	0
SE Boggy	5	4	52	2	3
SE Kiamichi	5	3	13	0	0
SE Little	13	7	29	1	2
Total	28	19	112	3	5
		(67.9)	(5.9)	(15.8)	(4.5)
NW total	64	40	203	2	3
		(62.5)	(5.1)	(5.0)	(1.5)
SE total	119	72	407	10	21
		(60.5)	(5.7)	(13.9)	(5.2)
Grand total	183	112	610	12	24
		(61.2)	(5.4)	(10.7)	(3.9)

[a] regions defined by the 3400 heating degree day (HDD) cline bisecting Oklahoma from northeast to southwest.

Table 2. Allele frequencies at three loci and average of combined frequencies for alleles of Florida sub-specific origin (F) for largemouth bass collected in 1992 and 1993 in Oklahoma streams.

Region[a]/Drainage (# individuals)	sIDHP			sAAT-B				sSOD		Average[b]
	*0	*1	*3	*1	*2	*3	*4	*1	*2	(F)
Arkansas:										
NW/Cimarron (33)	0.000	1.000	0.000	0.985	0.015	0.000	0.000	0.000	1.000	0.0000
NW/Salt Fork (22)	0.000	1.000	0.000	0.864	0.136	0.000	0.000	0.000	1.000	0.0000
NW/Verdigris (27)	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.0000
NW/Neosho (37)	0.000	0.986	0.014	1.000	0.000	0.000	0.000	0.000	1.000	0.0047
SE/Main Ark. (32)	0.000	1.000	0.000	0.953	0.031	0.016	0.000	0.016	0.984	0.0053
SE/Illinois (26)	0.000	0.962	0.038	0.827	0.154	0.019	0.000	0.019	0.981	0.0253
SE/Poteau (30)	0.000	0.983	0.017	0.900	0.083	0.017	0.000	0.000	1.000	0.0113
N. Canadian:										
NW/w. of OKC (30)	0.000	1.000	0.000	0.900	0.100	0.000	0.000	0.000	1.000	0.0000
SE/e. of OKC (38)	0.000	1.000	0.000	0.868	0.132	0.000	0.000	0.000	1.000	0.0000
S. Canadian:										
NW/w. of OKC (12)	0.000	1.000	0.000	0.833	0.167	0.000	0.000	0.000	1.000	0.0000
SE/e. of OKC (43)	0.000	1.000	0.000	0.884	0.116	0.000	0.000	0.000	1.000	0.0000
Red River west of Texoma Dam:										
NW/Washita w OKC(22)	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.0000
NW/North Fork (20)	0.000	0.950	0.050[c]	0.975	0.000	0.025	0.000	0.000	1.000	0.0250
SE/Washita e OKC(40)	0.000	0.987	0.012	0.900	0.100	0.000	0.000	0.000	1.000	0.0040
SE/Cache-Beaver (18)	0.000	0.694	0.306	0.778	0.000	0.194	0.028	0.000	1.000	0.1760
SE/Wichita Mt. (54)	0.000	1.000	0.000	0.935	0.065	0.000	0.000	0.000	1.000	0.0000
SE/Brier-Caney (14)	0.000	1.000	0.000	0.893	0.107	0.000	0.000	0.000	1.000	0.0000
Red River east of Texoma Dam:										
SE/Blue (18)	0.056	0.944	0.000[c]	0.917	0.083	0.000	0.000	0.000	1.000	0.0000
SE/Boggy (52)	0.010	0.981	0.010	0.942	0.019	0.029	0.010[c]	0.010	0.990	0.0197
SE/Kiamichi (13)	0.000	1.000	0.000	0.769	0.231	0.000	0.000	0.000	1.000	0.0000
SE/Little (29)	0.000	0.948	0.052[c]	0.948	0.017	0.034	0.000[c]	0.000	1.000	0.0287

[a] regions defined by the 3400 heating degree day (HDD) cline bisecting Oklahoma

[b] Average F = [f(sIDHP*3) + f(sAAT-B*3 + *4) + f(sSOD*1)] / 3

[c] does not conform to HWE (Fisher's exact test, $p < .05$)

Table 3. Sites in Oklahoma in 1992-1993 where bass (LMB) carrying Florid alleles (FLMB) were collected and number of FLMB collected. Columns inidcate number of Florida, F_1, F_x intergrades and total bass with Flori alleles.

River drainage site, nearest town	FLMB	Intergrade F_1	Intergrade F_x	TBFA
Neosho:				
Big Cabin Creek, Vinita			1	1
Arkansas:				
Georges Fork, Warner			1	1
Illinois:				
Sayer Creek, W. Siloam Springs			3	3
Poteau:				
Unnamed tributary south of Calhoun		1		1
Washita:				
Big Sandy Creek, Tishomingo			1	1
North Fork of the Red:				
Lake Creek, Granite			2	2
West Cache Creek:				
Blue Beaver Creek, Lawton			1	1
Crater Creek, Cache			3	3
West Cache Creek, Cache		5	1	6
Muddy Boggy River:				
Sand Creek, Atoka			1	1
Clear Boggy River:				
Sandy Creek, Filmore			2	2
Little River:				
Little River, Wright City	1		1	2
Totals	1	6	17	24

Table 4. Allele frequencies at three selected loci for largemouth bass collected in 1992-1995 in Oklahoma streams. All populations were fixed for a single allele at sMDH-A and GPI-A.

Region/Drainage (# individuals)	sMDH-B			PGM			GPI-B	
	*1	*2	*3	*2	*3	*4	*2	*3
Arkansas:								
NW/Cimmarron (33)	0.485	0.515	0.000	0.091	0.879	0.030	1.000	0.000
NW/Salt Fork (22)	0.477	0.523	0.000	0.045	0.955	0.000	1.000	0.000
NW/Verdigris (27)	0.685	0.315	0.000	0.000	1.000	0.000	1.000	0.000
NW/Neosho (37)	0.581	0.419	0.000	0.000	1.000	0.000	1.000	0.000
SE/Main Ark. (32)	0.469	0.531	0.000	0.000	1.000	0.000	0.984	0.016
SE/Illinois (26)	0.635	0.308	0.058[a]	0.000	1.000	0.000	0.865	0.135
SE/Poteau (30)	0.500	0.500	0.000	0.050	0.950	0.000	0.733	0.267
N. Canadian:								
NW/west of OKC (30)	0.517	0.483	0.000	0.000	1.000	0.000	1.000	0.000
SE/east of OKC (38)	0.737	0.263	0.000	0.000	1.000	0.000	0.947	0.053
S. Canadian:								
NW/west of OKC (12)	0.417	0.583	0.000	0.042	0.958	0.000	1.000	0.000
SE/east of OKC (43)	0.709	0.291	0.000	0.000	1.000	0.000	1.000	0.000
Red River west of Texoma Dam:								
NW/Washita w OKC(22)	0.795	0.205	0.000	0.000	1.000	0.000	1.000	0.000
NW/North Fork (20)	0.650	0.350	0.000	0.025	0.925	0.050[a]	0.900	0.100
SE/Washita e OKC (40)	0.675	0.325	0.000	0.000	1.000	0.000	0.938	0.063
SE/Cache-Beaver (18)	0.472	0.528	0.000	0.000	1.000	0.000	0.889	0.111
SE/Wichita Mts. (54)	0.491	0.509	0.000	0.000	1.000	0.000	0.991	0.009
SE/Brier-Caney (14)	0.607	0.393	0.000	0.000	1.000	0.000	0.964	0.036
Red River east of Texoma Dam:								
SE/Blue (18)	0.500	0.500	0.000	0.000	1.000	0.000	1.000	0.000
SE/Boggy (52)	0.452	0.548	0.000	0.029	0.971	0.000	1.000	0.000
SE/Kiamichi (13)	0.308	0.692	0.000	0.000	1.000	0.000	1.000	0.000
SE/Little (29)	0.259	0.741	0.000	0.000	1.000	0.000	1.000	0.000
NW average	0.576	0.424	0.000	0.025	0.965	0.010	0.987	0.013
SE average	0.524	0.472	0.004	0.006	0.994	0.000	0.947	0.053

[a] alleles at this locus do not conform to HWE (Fisher's exact test, $p < .05$)

Table 5. Genetic variability in largemouth bass from streams sampled in Oklahoma 1992-1993.

Region[a]/Drainage	Mean number of alleles per locus (SE)	Percentage of loci polymorphic	Mean heterozygosity	
			Direct Count (SE)	Expected[b] (SE)
Arkansas River:				
NW Cimarron	1.5 (0.3)	37.5	0.087 (0.057)	0.095 (0.065)
NW Salt Fork	1.4 (0.2)	37.5	0.085 (0.052)	0.105 (0.065)
NW Verdigris	1.1 (0.1)	12.5	0.051 (0.051)	0.055 (0.055)
NW Neosho[c]	1.3 (0.2)	25.3	0.068 (0.064)	0.065 (0.061)
SE Main Arkansas tribs[c]	1.6 (0.3)	50.0	0.082 (0.061)	0.083 (0.062)
SE Illinois[c]	1.9 (0.3)	62.5	0.135 (0.056)	0.145 (0.066)
SE Poteau[c]	1.8 (0.3)	62.5	0.150 (0.071)	0.153 (0.070)
Canadian River:				
NW North Canadian west of OKC	1.3 (0.2)	25.0	0.096 (0.072)	0.086 (0.064)
NW South Canadian west of OKC	1.4 (0.2)	37.5	0.135 (0.086)	0.110 (0.067)
SE North Canadian east of OKC	1.4 (0.2)	37.5	0.099 (0.057)	0.091 (0.052)
SE South Canadian east of OKC	1.3 (0.2)	25.0	0.090 (0.064)	0.078 (0.055)
Red River west of Lake Texoma Dam:				
NW Washita west of OKC	1.1 (0.1)	12.5	0.028 (0.028)	0.042 (0.042)
NW North Fork and western tribs[c]	1.8 (0.3)	62.5	0.119 (0.073)	0.118 (0.056)
SE Washita east of OKC[c]	1.5 (0.2)	50.0	0.106 (0.062)	0.096 (0.055)
SE Cache and Beaver Creeks[c]	1.6 (0.3)	50.0	0.215 (0.090)	0.190 (0.078)
SE Wichita Mt. Wildlife Refuge	1.4 (0.2)	37.5	0.072 (0.057)	0.081 (0.062)
SE Brier and Caney Creeks	1.4 (0.2)	37.5	0.098 (0.063)	0.096 (0.062)
Red River east of Lake Texoma Dam:				
SE Blue	1.4 (0.2)	37.5	0.090 (0.070)	0.097 (0.063)
SE Boggy[c]	2.0 (0.4)	62.5	0.082 (0.053)	0.091 (0.060)
SE Kiamichi	1.3 (0.2)	25.0	0.096 (0.065)	0.102 (0.067)
SE Little[c]	1.5 (0.3)	37.5	0.056 (0.047)	0.074 (0.048)
NW Mean	1.4 (0.2)	31.3 (16.4)	0.084 (0.035)	-----
SE Mean	1.5 (0.2)	44.2 (13.1)	0.105 (0.041)	-----
Non-Florida[d] Mean	1.3 (0.1)	30.2 (9.9)	0.086 (0.027)	-----
Florida[c] Mean	1.6 (0.2)	51.4 (13.1)	0.113 (0.049)	-----

[a] defined by the 3400 heating degree day (HDD) cline bisecting Oklahoma from northeast to southwest.
[b] unbiased Hardy-Weinberg estimate (see Nei 1978)
[c] Florida sub-specific alleles detected within the drainage
[d] Florida sub-specific alleles not detected within the drainage

Table 6. Genetic differentiation of Oklahoma largemouth bass drainage populations in 1992-1993 within large rivers (Arkansas, Canadian, Red River East and West of Oklahoma City), major drainages (Arkansas and Re River) and statewide.

	Index of genetic differentiation	
	Absolute	Relative
Comparison, x-y	σ_{xy}	F_{xy}
Drainage-large rivers	0.043	0.052
Drainage-major drainages	0.064	0.076
Drainage-statewide	0.050	0.060
Large rivers-major drainages	0.021	0.025
Large rivers-statewide	0.007	0.009
Major drainages-statewide	-0.013	-0.017

Table 7a. Matrix of Nei's (1978) unbiased genetic identity averaged by large river drainage.

large river	No. of populations	1	2	3	4
1 Main Arkansas	7	0.993 (0.983-1.000)			
2 Canadian	4	0.993 (0.981-1.000)	0.993 (0.984-1.000)		
3 West Red	6	0.992 (0.972-1.000)	0.992 (0.968-1.000)	0.989 (0.961-1.000)	
4 East Red	4	0.989 (0.965-1.000)	0.987 (0.95601.000)	0.983 (0.949-1.000)	0.994 (0.991-1.000)

Table 7b. Matrix of Nei's (1978) unbiased genetic identity for Oklahoma largemouth bass averaged by major drainage 1992-1993.

major drainage	No. of populations	1	2
1 Arkansas	11	0.993 (0.981-1.000)	
2 Red	10	0.990 (0.956-1.000)	0.986 (0.949-1.000)

Table 8. Minimum-length spanning tree for drainage populations of largemouth bass in Oklahoma 1992-1993.

Drainages connected	Length
Cimmaron - Salt Fork	.02732
Salt Fork - S.W. Canadian	.00093
Salt Fork - Boggy	.00951
Boggy - Neosho	.01115
Neosho - Verdigris	.00197
Verdigris - W. Washita	.00211
Verdigris - E. Wahsita	.00216
E. Washita - S.E. Canadian	.00094
S.E. Canadian - N.E. Canadian	.00068
E. Washita - Brier	.00105
Brier - N.W. Canadian	.00177
N.W. Canadian - Wichita Mts.	.00036
Wichita Mts. - Main Arkansas	.00031
Wichita Mts. - Blue	.00062
Main Arkansas - Little	.00789
Salt Fork - Poteau	.01408
Little - Kiammichi	.02072
Blue - Cache	.02323
Boggy - N. Fk. Red	.03067
E. Washita - Illinois	.03503

Figure 1. Sites surveyed for largemouth bass in Oklahoma, 1992-1993. Stars show sites where Florida alleles were detected.

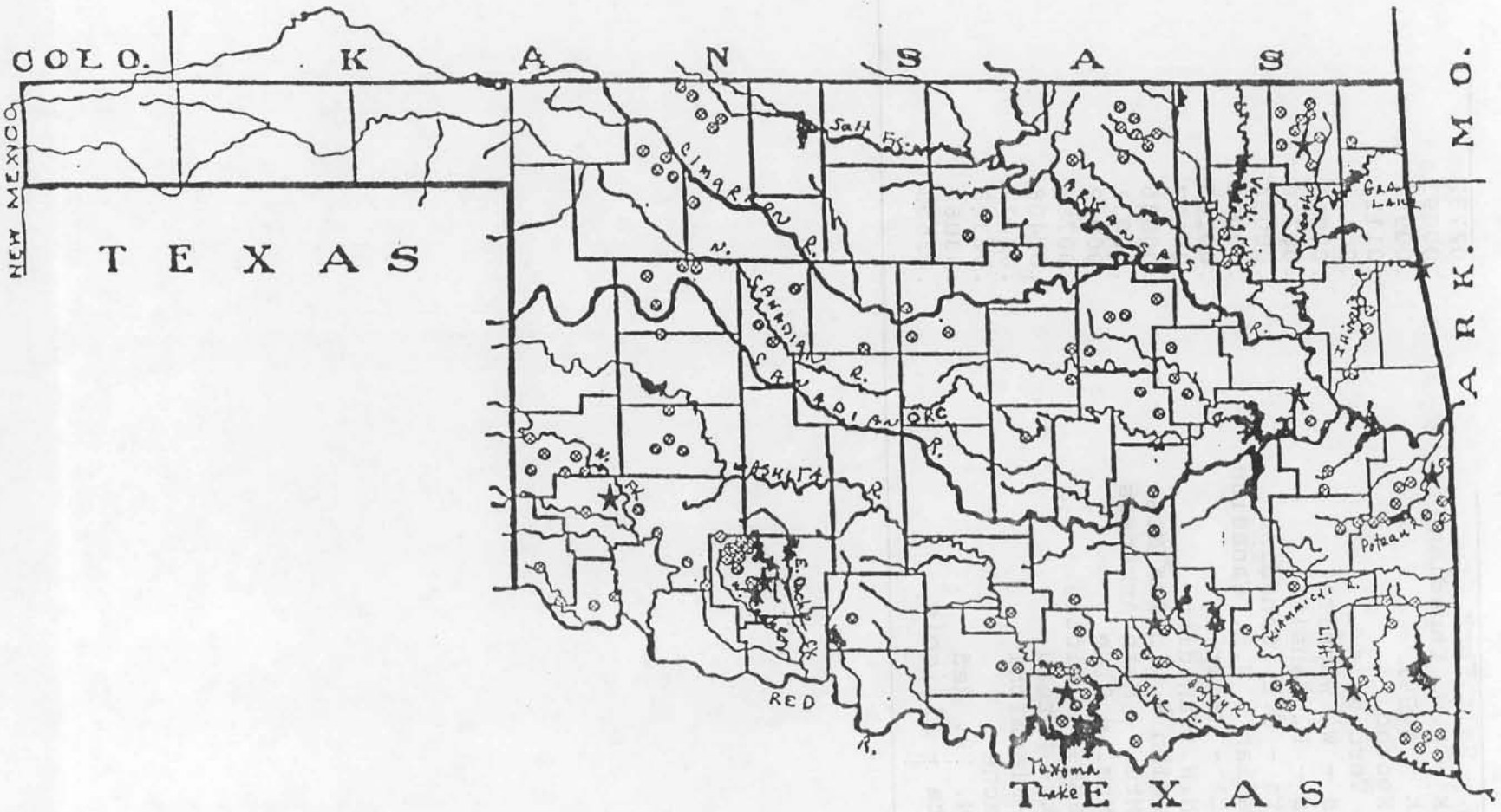

Figure 2. Cluster analysis of Oklahoma stream largemouth bass, 1992-1993, based on genet identity (Nei 1978) and UPGMA clustering method. Letter code for each drainage is shown. Scale indicates level at which each drainage entered the cluster.

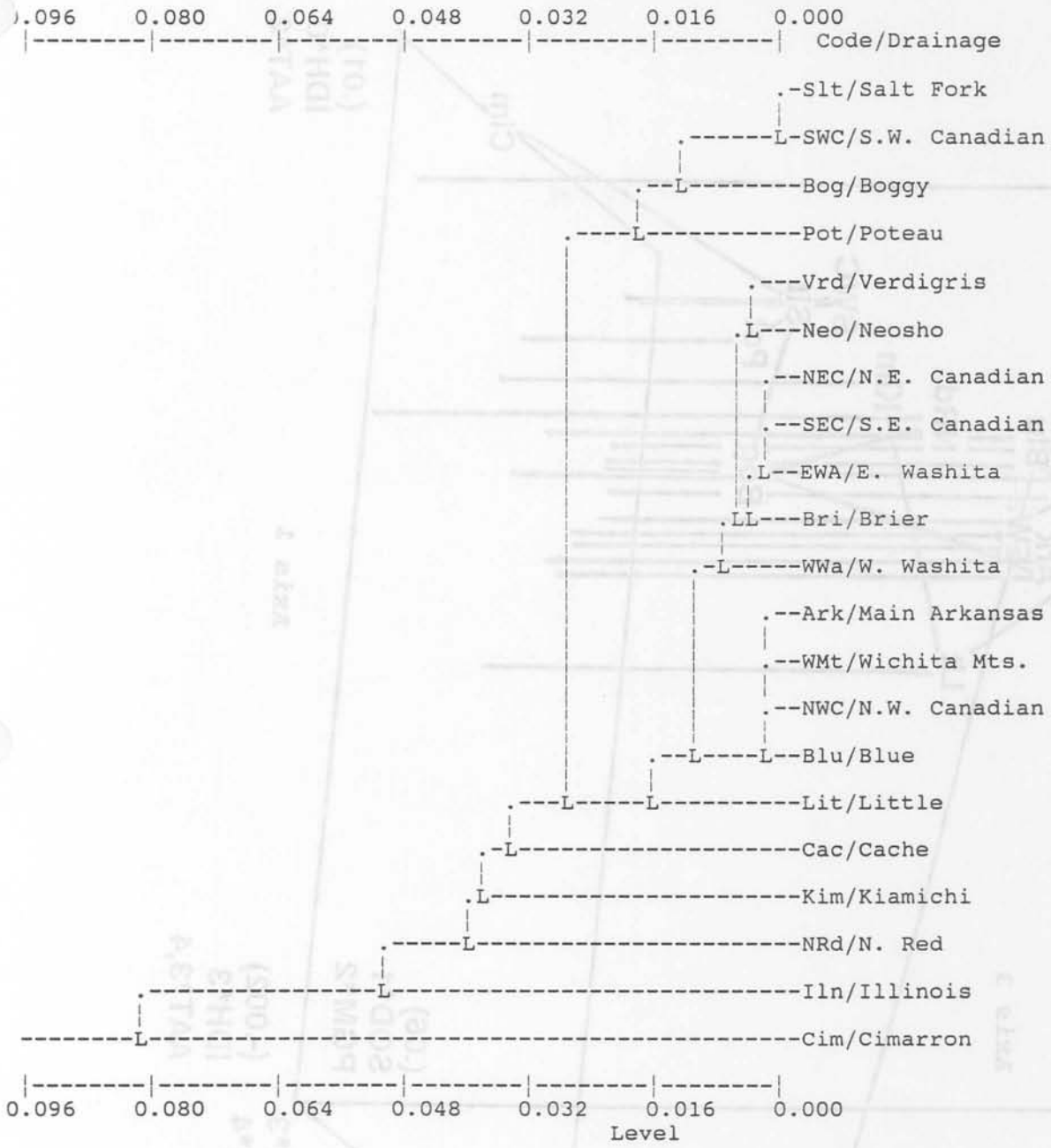
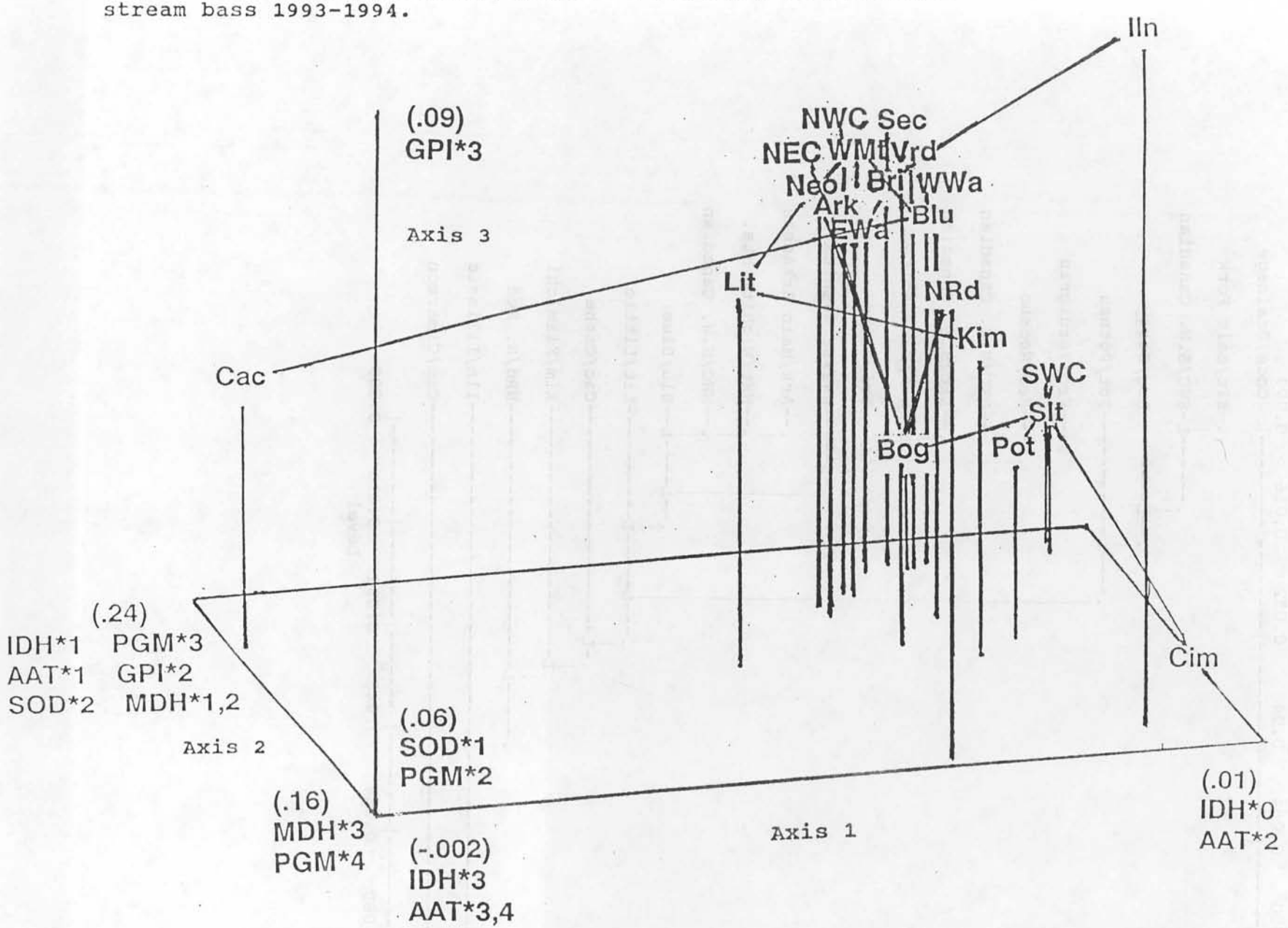
Figure 3. Ordination showing genetic similarity of Oklahoma stream bass 1993-1994.